

# Antimicrobial activity of seven metallic compounds against penicillinase producing and non-penicillinase producing strains of *Neisseria gonorrhoeae*

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**SUMMARY** The in vitro activity of seven metallic compounds was tested against penicillinase ( $\beta$  lactamase) producing strains of *Neisseria gonorrhoeae* (PPNG) and non-PPNG strains.

On a weight basis, the mercurials showed the greatest in vitro activity. Phenylmercuric borate, thiomersal, and mercuric chloride inhibited 90% of all strains at concentrations of 5 mg/l, 5 mg/l, and 20 mg/l respectively. Silver nitrate inhibited 90% of the strains at 80 mg/l and the MIC<sub>90</sub> for mild silver protein was 200 mg/l. Copper and selenium salts had lower in vitro activities, inhibiting 90% of all the strains at 320 mg/l and 640 mg/l respectively. Silver nitrate and the six other compounds tested showed equal activities against PPNG and non-PPNG strains. This finding supports the recommendation for prophylaxis of gonococcal conjunctivitis of the newborn with 1% silver nitrate eye drops.

## Introduction

Gonococcal ophthalmia neonatorum was a leading cause of childhood blindness before the penicillin era.<sup>1</sup> Prevention was essential. In 1881 Cr  d   introduced silver nitrate for prophylaxis, and its use became widespread. Where prophylaxis was implemented the incidence of blindness caused by gonococcal ophthalmia neonatorum decreased dramatically. The transient chemical conjunctivitis that occurs in up to half the of neonates given 1% silver nitrate eye drops caused reservations about the practice.<sup>2-3</sup> Since the 1960s some countries have switched from silver nitrate to antibiotics, such as tetracycline, erythromycin, or penicillin, for prophylaxis against neonatal ocular infection with *Neisseria gonorrhoeae*, whereas others abandoned prophylaxis.<sup>4</sup>

In 1976 infections caused by penicillinase producing strains of *Ngonorrhoeae* (PPNG) emerged and have been increasing since.<sup>5</sup> These PPNG strains render the treatment of gonococcal ophthalmia difficult as the appropriate antibiotics, such as cefotaxime or kanamycin,<sup>6-8</sup> are not widely available, particularly in developing countries. It is not known whether silver nitrate would be less effective in preventing

gonococcal ophthalmia caused by PPNG strains. In this study, we tested the in vitro activity of silver nitrate and six other antimicrobial metallic compounds against PPNG and non-PPNG strains.

## Materials and methods

We tested 58 strains of *Ngonorrhoeae*, 13 of which were PPNG as assessed by the chromogenic cephalosporin test.<sup>9</sup>

We measured minimum inhibitory concentrations by an agar dilution technique using GC agar base (Oxoid, Basingstoke, Hampshire, England) supplemented with 1% haemoglobin (Oxoid) and 1% IsoVitaleX (BBL). Using a multipoint replicator we inoculated suspensions of overnight cultures in 2 ml blood-saponin broth (tryptic soy broth (Difco Laboratories, Detroit), 1% saponin (Merck, Darmstadt, Germany), and 5% horse blood) on to plates containing twofold dilutions of the antimicrobials. The inoculum was 10<sup>5</sup> colony forming units (cfus). The plates were incubated for 24 hours at 35  C in an atmosphere of 5% carbon dioxide. The MIC was the lowest concentration of the antimicrobial that inhibited visible growth.

We tested the following antimicrobial products: silver nitrate (Merck, Darmstadt, Germany), mild silver proteinate (Cooper Lab, USA), mercuric chloride (Merck), thiomersal (Federa, Brussels, Belgium), phenylmercuric borate (Sigma, St Louis,

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Accepted for publication 19 September 1985

Missouri, USA) copper sulphate (Merck), and sodium selenite (Riedel-De Haen AG, Seelze-Hannover, Germany).

## Results

The table shows the MICs of the seven antimicrobial metallic compounds for the 58 strains of *Ngonorrhoeae*. All three mercurials were highly active against all strains tested. The MICs of thiomersal and phenylmercuric borate were comparable: 90% of the strains were inhibited by 5 mg/l thiomersal and 2.5 mg/l phenylmercuric borate respectively. The MIC of the inorganic mercury compound was higher than that of the organic compounds, probably because the free Hg<sup>++</sup> ions are preferentially bound to compounds of the very rich GC agar base medium. Silver nitrate showed a lower MIC<sub>90</sub> (80 mg/l) than mild silver proteinate (200 mg/l). Copper sulphate inhibited 90% of all strains at a concentration of 320 mg/l, and the MIC<sub>90</sub> for sodium selenite was 640 mg/l. In contrast, *Haemophilus ducreyi* has been shown to have a high susceptibility to sodium selenite (MIC 1 mg/l to 4 mg/l) and copper (II) chloride (MIC 2 mg/l to 8 mg/l).<sup>9</sup> All the products tested except sodium selenite had a very narrow range of MICs.

For all the antimicrobial metallic compounds tested, there was no difference in susceptibility between PPNG and non-PPNG strains.

## Discussion

The increasing prevalence of PPNG strains, particularly in developing countries, makes it important to prevent neonatal conjunctivitis caused by *Ngonorrhoeae*.

TABLE Minimum inhibitory concentrations (MICs) of seven antimicrobials against 13 strains of penicillinase producing *Neisseria gonorrhoeae* (PPNG) and 45 non-PPNG strains

Product	Strain	Range of MICs (mg/l)	MIC <sub>50</sub> (mg/l)	MIC <sub>90</sub> (mg/l)
Silver nitrate	Non-PPNG	40 - 160	80	80
	PPNG	20 - 80	80	80
Mild silver proteinate	Non-PPNG	50 - 200	200	200
	PPNG	50 - 200	200	200
Mercuric chloride	Non-PPNG	10 - 20	10	20
	PPNG	10 - 20	10	20
Thiomersal	Non-PPNG	1.25- 5	2.5	5
	PPNG	1.25- 5	2.5	5
Phenylmercuric borate	Non-PPNG	0.6 - 2.5	2.5	2.5
	PPNG	1.25- 5	2.5	5
Sodium selenite	Non-PPNG	20 - 1280	320	640
	PPNG	20 - 640	320	640
Copper sulphate	Non-PPNG	80 - 320	320	320
	PPNG	320	320	320

This study shows that silver nitrate prophylaxis should be effective against PPNG and non-PPNG strains. Ophthalmic ointments containing 1% tetracycline or 0.5% erythromycin have been proposed as alternatives to the 1% silver nitrate eye drops.<sup>10</sup> It is uncertain whether these agents are as effective as silver nitrate in areas with increasing penicillin resistance. It has been shown that PPNG strains can also be resistant to several other antibiotics, including tetracycline and erythromycin,<sup>11 12</sup> but not whether this in vitro resistance affects the effectiveness of topical treatment. Moreover, in developing countries keeping fresh preparations of antibiotics for ophthalmic use is difficult. For these reasons prophylaxis with silver nitrate 1% eye drops immediately after birth has been recommended in areas with considerable risk of gonococcal ophthalmia neonatorum.<sup>7 13</sup> An alternative prophylactic method should be effective against all gonococcal strains, particularly PPNG strains; it should have no side effects, or fewer than silver nitrate; and it should be inexpensive. Such an alternative is in fact not available. We tested the in vitro activities of other metallic compounds on *Ngonorrhoeae*. This study showed that several metallic compounds, in particular the organic mercurials, are highly active in vitro against *Ngonorrhoeae*. Further studies are required to assess their activity against other micro-organisms, such as *Chlamydia trachomatis*, their cytotoxicity, and their irritating effect on the conjunctivae.

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